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THE FUNCTION OF INVERTASE IN THE FORMATION OF CANE AND INVERT SUGAR DATES

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The chemical character of the ovulary of the date, *Phoenix dactylifera*, has seldom been studied, although the seed has long furnished a convenient and favorite material for the most diverse researches. Published analyses with few exceptions have been made by the usual WEEENDE methods and consequently give no idea of the real chemical nature of the fruit. A French analysis¹ made before 1867 mentions

	Per cent.
Water	43.6
Albuminoid and pectin bodies	2.9
Gallic acid and glucose	47.9
Inulin	trace
Fat	0.4
Cellulose	1.9
Mineral matter	3.3

a trace of inulin, but otherwise is of little significance.

SENY² seemed to strengthen the prevalent view, that dates contain little or no cane sugar, by the analysis of a number of Mesopotamian dates which contained dextrose (invert sugar) only. In reply to SENY's paper, however, LINDET³ points out that a sample of Tunis dates—probably Deglet Noors which occur on the Paris market—analyzed by him in 1891 contains 23 per cent. of glucose (invert sugar) and 38 per cent. of cane sugar. This is the first mention of a cane sugar date that I have found, aside from recent comparative analyses, made at this station, of Arizona-grown dates from trees imported from all the date-growing regions of the world by the U. S. Department of Agriculture and placed in the Cooperative Date Orchard at Tempe, Arizona.

LINDET cautions against generalizing from a few analyses when they concern plants and fruits, for the absolute and relative proportions of sugars will vary with climate, condition of season, degree of maturity, etc. In this paper I intend to show that other factors,

¹ Recueil de Mémoires de Médecine de Chirurgie et de Pharmacie Militaires.

² Journal de la distillerie française. 1895.

³ *Ibid.* 118. 1895 [abstract Chem. Zeit. 20:898].

especially the presence of invertase, and probably limitations in the distribution of the same, are more potent in determining the relative proportion of the sugars than the factors mentioned by LINDET. According to observations on the date, it seems that fruits of the same species but of different chemical nature are true chemical mutants, determined by the plant itself rather than by its environment. Further investigations may reveal cane sugar varieties of what BUIGNET⁴ considered inverts sugar fruits, as grapes, currants, and figs, or invert varieties, among the partially cane sugar fruits, bananas, apricots, peaches, plums, apples, and pears.

SLADE,⁵ basing a classification on the analyses of varieties, from the Tempe orchard, of various native seedlings and of native fruits from Mexico, divided dates into two main classes: cane sugar dates, embracing the Deglet Noor, and M'Kentichi Degla; and invert sugar dates, embracing Rhars, native seedlings, Mexican dates, and most others. It is possible to have partial cane sugar dates, and such do exist, as I shall point out later. While SLADE's classification is correct for mature and cured dates, nevertheless all dates are decidedly of the cane sugar type at some time of their life-history.⁶ This fact gave the first clue to the influence of invertase in determining the saccharine character of the date and probably of most other fruits.

It appears probable that the carbohydrates may enter the fruit as cane sugar, for nearly all fruits, either cane or invert sugar, show an appreciable percentage of cane sugar at the period of maximum accumulation of dry matter. This is often very inconsiderable, as we see in KEIM's⁷ analysis of ripening cherries, and may not be detected at all, as in the ripening persimmon.⁸ Even in these cases more cane sugar would be found if special precautions were taken to destroy the invertase immediately after removing from the tree. The cane sugar of the date vanishes very rapidly in the invert varieties. MIERAN⁹

⁴ Compt. Rend. 51:894. 1860; through Bull. 94, Bur. of Chem., U. S. Dept. Agric.

⁵ All notes on the unfinished work with dates of H. B. SLADE (died June 5, 1905) were turned over to the writer and acknowledgment is made in this paper whenever material from that source has been used.

⁶ Ann. Rep. Arizona Agr. Exp. Sta. 17:164, 165.

⁷ Zeit. Anal. Chem. 30:401. 1891.

⁸ BIGELOW, GORE, and HOWARD. Jour. Am. Chem. Soc. 28:688.

⁹ Chem. Zeit. 56:1003, 1021, 1283. 1893.

found also that banana pulp not only reduced its own cane sugar rapidly, but could invert large amounts of added cane sugar. BAILEY,¹⁰ however, states that he failed to find any enzymic action in the ripening of the banana, but his figures show a dropping-off of cane sugar after the yellow-brown stage.

In a set of tests for invertase with very green, yellow, and black-ripe bananas, made in this laboratory, a feeble inverting power at room temperature was found in the two latter conditions, but not at all comparable with that of an invert sugar date under like conditions. It is possible that cane and invert sugar bananas may be found. In the banana, however, the reserve material in the fruit itself is largely starch and is finally converted into cane sugar, either directly or by way of maltose. Nevertheless, it would seem that some cane sugar, as such, may come into the banana ripened on the tree, for both BUIGNET¹¹ and RICCIARDI¹² agree that when ripened naturally this fruit contains more cane sugar and less invert sugar than when ripened artificially. Substantially the same is true with the cane sugar date. It is not untenable, however, that some other soluble carbohydrate may enter the fruit and be rapidly synthesized to cane sugar, for BROWN and MORRIS¹³ have shown that the excised barley embryo, cultivated on maltose solution, formed cane sugar very rapidly by the "action of the living embryo cell." They found also that living embryos did not synthesize cane sugar from glucose. This difficulty is not insurmountable, for HILL¹⁴ demonstrated the reversible nature of enzymic action by synthesizing maltose from glucose in very dilute solution by the aid of maltase. The maltose formed was later found to be isomaltose,¹⁵ but that does not necessarily mean that, under cell conditions, true maltose would not be formed. The reverse action of enzymes has been shown to be influenced, at least with regard to speed, by the presence of a third body. Thus ethylbutyrate is formed more rapidly by lipase in the presence of lecithin.¹⁶

¹⁰ Jour. Biol. Chem. **1**:355. 1906.

¹¹ Compt. Rend. **49**:276-278. 1859; through Bull. 94, Bur. of Chem.

¹² Compt. Rend. **95**:393. 1882; *ibid.*

¹³ Jour. Lond. Chem. Soc. **57**:458 (517). 1890.

¹⁴ Jour. Lond. Chem. Soc. **73**:634. 1898.

¹⁵ Through LOEB, *Dynamics of living matter* **11**. New York 1906.

¹⁶ HEWLETT, *ibid.* p. 10.

That cane sugar may find its way directly into the fruit would seem probable, moreover, since it is one of the most diffusible carbohydrates, and BROWN and MORRIS¹⁷ believe it the primary carbohydrate synthesized by the chloroplasts. Cane sugar, however, is not suited to nourish the protoplasm. If injected directly into the blood of animals, BERNARD¹⁸ has shown it to be eliminated unchanged by the kidneys, and to be inverted in the beet root before being used. Glucose appears in the beet root and can be traced up the stem, but not so with cane sugar. FORBES¹⁹ finds the same true with canaigre, *Rumex hymenosepalus*. The small amount of cane sugar contained in the dormant root suddenly decreases when the root begins to send up a stalk. The much larger amount of starch is similarly affected. As is well known, the moving carbohydrate of the maple is nearly pure cane sugar. Plant leaves are also known to contain invertase in conjunction with cane sugar. BROWN and MORRIS consider the entire amount of cane sugar to be hydrolyzed, first for the nutrition of the tissues, and then any excess to be resynthesized into starch for future use, the hydrolytic product of this starch being maltose. It appears thus that either cane sugar, invert sugar, or maltose has the opportunity of leaving the leaf. SAPOSCHNIKOFF²⁰ says the form in which the carbohydrate leaves the leaf is unknown, but is probably glucose.

It has been established, on the other hand, that the existence of starch in the tuber of the potato,²¹ in maize,²² and in wheat²³ depends on the previous existence of cane sugar in the juice of these plants. KEIM,²⁴ in HILGER's laboratory, found cane sugar to accumulate in the leaves of the cherry and starch in the fruit stems during the growing stage, both of which disappear at the period of maximum ripening, when a small amount of very transient cane sugar is found in the

¹⁷ A contribution to the chemistry and physiology of foliage leaves. *Jour. Lond. Chem. Soc.* **63**:604. 1893.

¹⁸ GREEN, The soluble ferments 110. Cambridge 1899.

¹⁹ Unpublished papers.

²⁰ Bildung und Wanderung der Kohlenhydrate in der Laubblättern. *Ber. Deutsch. Bot. Gesells.* **8**:233. 1890.

²¹ GIRARD, *Compt. Rend.* **108**:602. 1889.

²² ZEPLEY, *Compt. Rend.* **94**:1033. 1882.

²³ BALLAND, *Compt. Rend.* **106**:1610. 1888.

²⁴ *Loc. cit.*

fruit. They explained this erroneously by considering that the acidity of the leaf was insufficient to invert the cane sugar, and only after it reached the acid fruit could such inversion take place. The occurrence of some cane sugar in the earliest stages of growth of the cherry, and its subsequent disappearance, they also attribute to a lack of acid. BUIGNET²⁵ attempts to explain the high cane sugar content of some strawberries in much the same way, on the hypothesis that the sugar and acid exist in different cells, and that in the watery varieties diffusion and consequently inversion take place more rapidly than in drier berries. In a later paper²⁶ he concludes that acidity has nothing to do with the inversion, but that it is probably the work of a "nitrogenous ferment." In the date acidity has nothing to do with inversion, for the cane sugar dates are usually the more acid. Neither does the high acidity of the orange appear to determine inversion, for here the cane sugar increases during ripening, while the invert sugar remains nearly constant. This BERTHELOT and BUIGNET²⁷ think is especially remarkable, since the green fruit contains no starch from which to derive the cane sugar. The phenomenon, however, is almost identical with that of the cane sugar date and just what would be expected of any fruit having similar invertase relations. The accumulation of starch in the stem of fruits which contain none appears to be quite common and has been observed in the grape.²⁸ Whether or not this is the case with the date has not been determined.

While the observed fact that cane sugar accumulates at the time of maximum ripening in nearly all fruits, even the pea²⁹ and cucurbits,³⁰ points very strongly to that sugar as the original carbohydrate to enter the fruit, one point militates most powerfully against such a hypothesis; the partial osmotic pressure of cane sugar within the date would stop flow in that direction long before the high observed

²⁵ Résumé, Compt. Rend. **49**:276-278. 1859; through Bull. 94, Bur. of Chem.

²⁶ Compt. Rend. **51**:894. 1860; *ibid.*

²⁷ Compt. Rend. **51**:1094. 1860; *ibid.*

²⁸ FAMINTZIN, Ann. Oenol. **2**:242. 1871; and HILGER, Landw. Vers. Station **17**:245. 1874; through KEIM, *loc. cit.*

²⁹ SCHWARZ AND RIECHEN, Zeits. Unters. Nahr. Genuss. **1904**:550; and FRE-RICHS AND RODENBERG, Arch. Pharm. **243**:276. 1905.

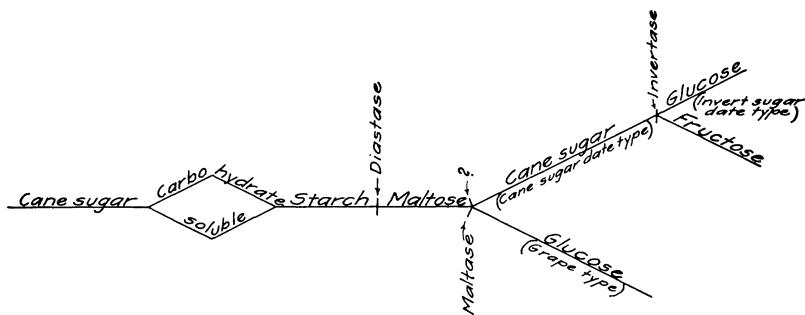
³⁰ LECLERC DU SABLON, Compt. Rend. **140**:320, 321. 1905.

percentages of cane sugar could be approached. The same is true also for glucose and fructose, for on the inversion of the cane sugar present they appear in approximately molecular proportions as determined by BROWNE'S³¹ method. Occasional samples vary considerably from this relation, giving sometimes a large excess of fructose, sometimes of glucose. This may be explained in part by a number of possibilities. The difficulties of technic in taking consecutive representative samples of such material as pulped date must be taken into consideration because data from two different samples enter the BROWNE formula. An excess of fructose may be accounted for by the hydrolysis of inulin. SLADE found inulase in some dates but has left no record of having found inulin. Neither inulin nor inulase was found by the writer in a sample examined in that regard. An excess of fructose over glucose might also originate in the preferential use of glucose by the tissues, as is known often to be the case. An excess of glucose might come from maltose and SLADE observed maltase, but I have not yet examined the date for that enzyme. Here again an excess of glucose might be consumed by the tissues, leaving nearly pure invert sugar. The relation between fructose and glucose in other fruits has never been worked out, but it appears that in the case of the date nearly all the sugar is cane sugar or a derivative of cane sugar.

Times thus undoubtedly occur with every individual when the partial osmotic pressure of these three sugars is approximately equal; nevertheless, accumulation of total sugars goes on rapidly. The date, then, as a species at least, must be prepared to pass in carbohydrate against a relatively high osmotic pressure of cane sugar, glucose, or fructose, either singly or simultaneously. Unless we choose to hold that the intercellular threads of living protoplasm pass along colloids and crystalloids against osmotic pressure, we are forced to seek some likely soluble carbohydrate against which there exists no considerable pressure. This place seems to be filled by maltose. So far as I am aware, neither the presence nor the absence of maltose has been demonstrated in the growing date. We should never expect to find any great amount, and it might be so transient as to escape detection entirely.

³¹ Bur. of Chem. Bull. 90: 10; also Jour. Am. Chem. Soc. 28: 439. 1906.

The following scheme for the translocation of sugars from stems and leaves to fruit seems then to be in accord with all known facts and contradicted by none. In the early stages cane sugar or mixed sugars move into the fruit until osmotic equilibrium is reached. After that, excess of cane sugar may be stored as starch within the fruit, as in the case of the banana and apple, thus diminishing the osmotic pressure for that sugar; or a part may be reduced to invert sugar, farther reducing the cane sugar pressure. On ripening, the starch is again transformed to soluble sugars. In those cases where no starch occurs in the fruit itself, but in the stem, the latter is probably a laboratory and not an ordinary storage tissue. The plant would scarcely have found it advantageous to thrust storage duties on the



stem when the fruit was fully capable and destined to receive the same material later. It seems more probable that after osmotic equilibrium is reached between the fruit and sap, those sugars against which there is osmotic pressure would be changed to starch at the threshold, and this starch in turn hydrolyzed by an amylase into maltose. Maltose, against which there is little or no osmotic pressure, would enter the fruit and there be split up into glucose or rearranged into cane sugar, which would be inverted where it comes into contact with invertase. Probably all these reactions take place to a greater or less degree in any ripening fruit, but the predominance of one or the other leads to a special type. If the main reaction were maltose to glucose, fruits of the grape type would result. If maltase secretion were suppressed, the maltose would follow the transformation it takes in fruits of the date type.

It is true that no catalytic outside the living cell is known which will transform maltose into cane sugar, but the cell is known to do so and the catalytic will probably be discovered. The transformation of starch to cane sugar seems to be established for the banana and apple, but here again no catalytic is known that will cause the transformation, although many, and some of organic origin, transform it into maltose. BROWN and MORRIS, moreover, have shown maltose to be the final product of starch hydrolysis in the leaf. Thus it seems probable that in these cases starch changes to cane sugar through maltose. It is also probable that the speed of transformation, maltose to sucrose, is very considerable, since BIGELOW and GORE³² find the curve representing the increase of cane sugar in a ripening apple to be nearly the reverse of that representing the decrease in starch.

An examination of the accompanying table will reveal the chief points of interest in the development of the date, and also the divergence between the cane and invert sugar varieties. The cane sugar date is represented by the fruits of the Deglet Noor³³ from palms imported from the oasis of French Algeria in 1900. The invert sugar date is from a seedling palm of excellent quality growing upon the university campus and immediately accessible from the laboratory.

It seems that any attempt to interpret a series of date analyses, made at different stages of growth, by reducing them to the dry basis, can give only a partial idea of what is actually taking place. Water has as much to do with the changes as cane sugar or invert sugar. A date having 20 per cent. of dry matter may change in a few days to one of 60 per cent. in three ways: by the loss of water through transpiration with decrease in weight; by the addition of dry matter without loss of water, possibly some gain, and increase in weight; or by the addition of dry matter with loss of water and little or no change in weight. In the case of the cherry, KEIM has noted that the period of maximum increase in percentage of dry matter in the fruit is parallel with that of maximum increase in weight. In the peach³⁴ the increase in solids is fairly proportional to the increase in water. With the date there is a more or less parallel increase in percentage of dry matter and

³² Bull. 94, Bur. of Chem., U. S. Dept. Agric.

³³ SWINGLE, Bur. Plant Ind., U. S. Dept. Agric. Bull. 53: 33.

³⁴ BIGELOW and GORE, Bur. of Chem., U. S. Dept. Agric. Bull. 97: 12.

CANE SUGAR DATE; DEGLET NOOR—TEMPE, ARIZ.

Date	Condition of sample	Condition of bunch		Av. wt. seeds	Per cent. cane sugar	Per cent. fructose	Per cent. glucose
		Belated	"				
Sept. 18, 1906	Pea size.	1.15	0.145	1.25	1.99	2.62	3.34
" 18, 1906	Green, about half grown.	5.02	0.572	17.41	3.82	3.27	3.27
" 18, 1906	Slightly yellow.	5.00	0.905	13.01	5.14	2.90	3.20
Oct. 5, 1906	Green, half grown.	6.66	0.705	10.58	1.48	2.84	4.17
" 5, 1906	Green, yellow tinge.	8.22	0.968	11.77	3.78	2.06	3.76
" 5, 1906	Red.	9.08	1.079	10.82	35.34	15.62	4.39
Sept. 7, 1906	Change more marked.	6.40	0.902	15.10	23.50	5.05	3.00
" 7, 1906	Same bunch.	6.40	0.794	12.41	30.24	13.86	3.97
Oct. 17, 1906	None ripe.	12.66	0.911	29.71	13.83	2.80	3.46
" 2, 1905	Some soft on end.	8.84	0.636	7.20	12.57	2.93	2.94
" 31, 1905	Red, stored 5 days.	8.91	0.705	7.92	47.70	31.61	3.36
" 26, 1905	Red, considerable yellow.	8.45	0.638	7.55	21.62	2.55	2.79
" 26, 1905	Same bunch.	8.92	0.671	7.33	53.45	5.26	5.40
" 26, 1905	Slightly spotted.	8.31	0.604	7.27	56.20	31.74	7.42
" 26, 1905	About one-third soft.	8.53	0.679	7.96	59.69	36.70	5.65
" 27, 1905	Slightly ripened.	8.44	0.672	6.98	62.65	33.07	5.57
" 27, 1905	Apparently fully ripe.	9.00	0.632	6.98	63.66	25.00	9.86
" 31, 1905	Art. ripened 30 hours.	8.90	0.676	7.55	63.00	25.00	14.13
" 31, 1905	Art. ripened 5 days from red.	7.48	0.639	8.54	61.64	21.09	14.12
" 31, 1905	Same apparently inferior.	6.97	0.637	9.14	62.02	27.18	12.54
" 6, 1905	Art. ripened.	7.48	0.629	8.42	57.82	24.81	12.51
Oct. 10, 1906	One-half to two-thirds ripe after 5 days ripening by solar heat.	9.00	0.732	8.13	62.49	37.79	7.19
" 10, 1906	Ripe on tree.	7.32	0.572	7.83	69.98	38.84	6.60
" 21, 1906	Ripe on tree.	6.26	0.691	9.52	74.03	41.51	10.38
" 21, 1906	Art. ripened, stored 21 days.	6.31	0.716	11.36	60.31	22.09	8.20
Dec. 4, 1906	Ripe on tree after frost.	8.19	0.643	8.41	73.20	44.71	14.83

CANE SUGAR DATE; DEGLET NOOR—HEBER, CALIFORNIA

Nov. 14, 1906	Ripe on tree.	INVERT SUGAR DATE; DEGLET NOOR—CAMPUS, TUCSON, ARIZ.		9.59	0.890	7.69	77.88	40.55	10.40	10.53
		10.54	0.788							
Oct. 3, 1906	Very green.	10.67	1.215	11.83	15.87	0.00	2.11	4.47		
" 10, 1906	Yellow.	1.120	1.047	31.77	7.95	8.01	0.99			
" 10, 1906	Yellow after cold week.	11.20	1.084	9.03	31.48	4.59	0.60			
" 10, 1906	Yellow, ripe in spots.	12.37	0.993	7.31	51.33	15.02	12.95	11.50		
" 19, 1905	Partly ripe, softened slightly.	12.18	1.057	45.07	11.56	13.86	13.46			
" 16, 1905	Partly brown.	11.38	0.767	7.58	51.37	10.01	16.81	15.90		
" 16, 1905	Ripe, not overripe.	12.07	0.997	10.19	52.36	6.18	21.02	20.08		
" 24, 1906	Ripe, still light brown.	12.97	0.997	7.51	55.14	6.76	16.07	15.70		
" 6, 1906	Ripe, stored 14 days.	10.97	0.927	55.29	0.28	23.87	24.91			
" 14, 1906	Ripe, stored 1 week.	9.34	0.707	8.21	57.99	1.42	25.05	24.25		

in weight (or size) till something like 20 per cent. of dry matter is present. Growth, so far as size is concerned, has then about reached its limit, but accumulation of dry matter in the form of sugar now takes place more rapidly, and in a relatively short time the dry matter rises from 20 or 25 per cent. to nearly 60 per cent. During this time there appears to be little or no further increase in weight. The rational interpretation then is that water is replaced by sugar. Curing again increases the dry matter up to 70 or even 85 per cent., but this change is purely a loss of water. These observations are further confirmed by purely practical ones. It may be observed that the individual dates on a bunch develop similarly throughout; the ones near the tips of the sprays remaining always somewhat smaller. At a certain period the bunch as a whole appears much the same and individuals begin ripening, but it may be a month or more before others on the same bunch, even on the same sprays, are ripe. The same thing is observed in artificial ripening experiments. Two sprays from the same bunch, and looking very much alike, will ripen under artificial conditions very differently. Some speedily develop into a plump, luscious fruit, while others dwindle away and finally furnish a thin skin of poor-quality flesh over an apparently normal seed. The seed seems to mature before marked changes in the ovulary begin. The fact that increase in dry matter takes place after the apparent maturity of the fruit must lie at the foundation of all economic attempts at artificial ripening. Any plans which ignore this will necessarily prove futile. The ripening process in the date, unlike that in the banana, is essentially one of addition and not of transformation. By reducing results to the dry basis, the very important economic as well as scientifically interesting observations made above would be entirely lost sight of. Furthermore, it would magnify any errors of technic from two to six times or more.

These objections cannot be raised, in like degree at least, to the method of expressing results by reducing percentages to absolute weight per date of each constituent at the several periods of development. It is evident, nevertheless, that this method also can give only a more or less distorted view, because it is practically impossible to select samples with any degree of assurance that they were of the same composition and weight as the previous sample at the time it was

taken. It seems more rational to consider the composition of each sample as we find it, together with its apparent condition of ripeness, the average weight of the individual fruits, the average weight and percentage of seeds, and finally the general relation which the individual fruits forming the sample seem to bear to the whole. By reduction to any other basis, some of these points, especially those involving personal equation in the greatest degree, are masked but not equalized.

Study of the analyses reveals a marked similarity in the composition of the two varieties up to the time of maturity in size. The Deglet Noor shows an inclination toward cane sugar even in the early stages, but from this point on it gains chiefly in cane sugar until ripeness is approached, when some inversion takes place. The amount of this inversion seems to be influenced largely by the temperature to which the ripening date is subjected. Under normal conditions, 20 to 25 per cent. of invert sugar is formed; but under the conditions necessary for artificial ripening, 45 to 50° C for several days, a much larger proportion is inverted. The sample ripened on the tree after frost shows less inversion. The invert sugar date shows an increase in invert sugar parallel to the increase in dry matter. At the period of maximum ripening, when dry matter is accumulating very rapidly, cane sugar appears in considerable quantities. This I attribute to the formation of cane sugar at a greater rate than the invertase is capable of inverting it. The same is also undoubtedly true with other invert sugar fruits, their sugar passing through cane sugar which is at times formed more rapidly than inverted. Many varieties of dates have been examined in this respect, and cane sugar is always observed to accumulate just before the date softens. At the time of softening the invertase, which before this cannot be dissolved by water or glycerin, is now readily extracted by these solvents. In the thick syrupy juice of the date this increased mobility of the invertase must greatly accelerate its action. This accounts for the more rapid disappearance of cane sugar after ripening.

The difference in behavior of the two classes of dates during ripening suggested that the presence or absence of invertase must be the determining factor, and accordingly, the inverting power of a Deglet Noor glycerin extract was tested as follows: 100^{cc} of date extract was

added to 2000^{cc} of 5 per cent. sugar solution, which was divided into portions of 200^{cc} each; one series, containing boiled and unboiled samples, was placed in an incubator at 49° C; the other was left at room temperature. After twenty-four hours no change could be noted in either series. It appeared that Deglet Noor contained no invertase.

A series of comparative extracts of Deglet Noor and three invert sugar dates was then prepared, using five parts of glycerin and seven parts of date, and pressing after digesting together for twenty-four hours or longer. In the tests the same quantities of 5 per cent. sugar solution and of date extract were used in each case. The preservative used throughout was thymol. The figures show the reading on the saccharimeter when portions of the solution were weighed off and treated in the usual manner. It will be noted that the inversion in the case of the three invert sugar dates follows, in a general way, the mass law as found by O'SULLIVAN and THOMPSON³⁵ for yeast invertase. In another series all the invert sugar date extracts were found to work better at 35° C, but the optimum was not determined.

INVERTING POWER OF DATE EXTRACTS

Date	Hour	Deglet Noor	Rhars	Birket el Haggi	Row 12, no. 7
Nov. 6	2 P. M.	5.14	4.51	4.47	4.49
" 6	5 P. M.	5.05	4.32	4.33	4.26
" 7	9 A. M.	5.17	3.36	2.92	3.05
" 7	4 P. M.	5.19	3.01	2.61	2.72
" 8	9 A. M.	5.18	0.81	1.13	1.04
" 9	9 A. M.	5.16	—1.69	—0.65	—1.41
" 10	9 A. M.	5.15	—1.77	—1.99	—1.58
" 12	9 A. M.	5.15	—1.92	—1.79	—1.76

The question then arose as to whether the Deglet Noor contained no invertase or whether an inhibiting substance was present. The first experiment had already shown that the failure to invert was not due to low temperature. Accordingly a series of tests with equal amounts of invert sugar date extract were arranged and increasing amounts of Deglet Noor extract added until 100^{cc} of the latter were present to 25^{cc} of the former. In no case could a diminution of the inverting power of the invert sugar date extract be observed. To make this matter more certain, a concentrated aqueous extract of

³⁵ Invertase, a contribution to the history of an enzyme or unorganized ferment. Jour. Lond. Chem. Soc. 57:834. 1890.

Deglet Noor was prepared and found to contain, per liter, 90.32^{gm} cane sugar, 18.81^{gm} fructose, and 17.78^{gm} glucose. A similar artificial mixture was made and both treated with equal amounts of Rhars extract. The natural Deglet Noor extract was inverted slightly faster than the artificial one. This proved that no antifermen was present.

While glycerin extracts of Deglet Noor and M'Kentichi Degla fail to invert solutions of cane sugar, the presence of considerable quantities of invert sugar in these dates points with certainty to invertase. It is untenable, moreover, to consider that these dates differ from the invert varieties merely in quantity of invertase without other qualifications, for a very small amount of invertase will invert a very great amount of cane sugar. O'SULLIVAN found that one part of crude invertase inverted 100,000 parts of cane sugar and still retained its inverting powers. It must be remembered also that a significant amount of invert sugar is formed in a very short time about the period of softening, and that this amount is greater under changed conditions, especially the higher temperature and moisture required for artificial ripening. The inverting action, however, is limited, for cane sugar determinations in samples one year old showed for Deglet Noor 42.14 per cent., M'Kentichi Degla 59.05 per cent., Saffraia 2.96 per cent. The same sample of Saffraia one year before contained nearly 4 per cent. cane sugar. This failure to invert is not due to the invertase becoming inactive, for a Rhars extract made from dates stored one year was fully as active as fresh date extract. A portion of the pulverized Saffraia used above, tested for inverting powers, proved to be one of the strongest preparations examined. These phenomena seem to be due to the localization of the invertase, which means suppression of invertase-secreting tissue. The only thing that could have prevented the inversion of the residual cane sugar in the Saffraia must have been inability to come into molecular contact with the enzyme. The tissues of all dates exhibit inverting powers, although the cane sugar varieties show it but feebly. The failure of their glycerin extracts to invert is probably due to the very small amount of invertase remaining in the same condition as it exists in the green invert sugar date.

Two ways suggest themselves for studying the distribution of the

invertase: testing various portions of the date for inverting power, and comparative analyses of the various parts. Suitable materials for carrying on these lines of work were exhausted this year before a fair start could be made. It is our expectation to continue this phase of the work. Preliminary tests by the first method—dividing the date transversely into blossom third, middle third, and stem third; and concentrically into three parts, the outer shell down to the tannin layer, the tannin layer, and the inner portion—failed to give trustworthy results.

The physiological function of the tannin is little understood. Whatever else it may do, it efficiently protects the fruit from animal ravages and permits it to mature its seed, as has been demonstrated in the Tempe orchard this year.³⁶ The loss of astringency on ripening was formerly believed to mark the conversion of tannin into sugar, but it was found that in the persimmon³⁷ the tannin only becomes insoluble. This has been found by THORNBER³⁸ and the writer to be true of the date also. SLADE considered the tannin present as glucoside which was in some way split up, the tannin being oxydized by an oxydase; and in fact some experiments in which the loss of astringency and softening appeared to have been hastened by the use of manganese salts gave some ground for this opinion. This was further supported by the observations of TICHOMIROV.³⁹ Even if true, the tannin or tannin-glucoside is so small in amount that it could not appreciably interfere in the carbohydrate relation. Many other quantitatively minor changes undoubtedly take place at the time of softening, among others the change in the behavior of the invertase toward solvents, which I hope to discuss at length in some future paper.

There remains at least one other way in which cane sugar dates differ chemically from invert sugar dates. E. E. FREE, who assisted in these experiments, noticed and called my attention to the fact that sugar solutions to which cane sugar date extracts had been added developed a pink tint. This was more pronounced and developed

³⁶ Annual Report, Ariz. Agric. Exp. Sta. 17:164.

³⁷ BIGELOW, GORE, and HOWARD, *loc. cit.*

³⁸ *Loc. cit.*

³⁹ Compt. Rend. 39:305. 1904; through notes of H. B. SLADE.

quicker in boiled solutions than in unboiled, and it was therefore not an enzyme reaction. We found oxygen essential to the color development, because solutions kept in an atmosphere of hydrogen over alkaline pyrogallol never turned pink, even in the bright sunlight, where they were heated to 50 or 55° C. Either in the dark or in the sunlight the color developed readily if free access of oxygen was allowed. This change was never noticed when using invert sugar date extracts. Whether or not it has any connection with the invertase is unknown.

Aside from the chemical classification into cane and invert sugar dates, there is also a physical one into dry and soft dates. We find both cane and invert sugar dates in the dry class as well as in the soft. The Deglet Noor is a typical cane sugar date, yet decidedly a soft date. The M'Kentichi Degla is also a cane sugar date, but of the hard class. The Saffraia, on the other hand, is as typical a hard date as the M'Kentichi Degla, but for all that practically an invert sugar date. The Halloua is a hard but only partial cane sugar date, somewhat similar in sugar content to the artificially ripened Deglet Noor, i. e., about 20 to 25 per cent. of cane sugar. In ripening, the hard dates do not soften or change color; they remain yellow. They shrivel somewhat, but this cannot be used as an index of ripeness, because the shriveling begins before the astringency disappears.

The origin of the cane and invert sugar dates is obscure, and the varieties have existed several hundred years. Nothing is known as to whether they originated from seedlings or were sports. They are now propagated only from suckers. It will prove interesting to study the seedlings to determine whether a cane sugar date can be obtained from a cane sugar date seed. This is an inviting field of investigation, but, due to the slow development of the plant, would require several generations of investigators.

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